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Electron microscopical morphometry of GH producing pituitary adenomas in comparison with normal GH cells*, **

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Summary. GH producing adenomas of patients with acromegaly (undifferentiated acidophil adenomas and well differentiated GH cell adenomas) were studied at the ultrastructural level and analysed morphometrically by the point counting method. They were compared with identically prepared GH cells of normal pituitaries from patients undergoing surgery for metastasizing cancer of the prostate. In the well differentiated GH cell adenomas significantly more points were counted on nucleoli, unorganized cytoplasm, rough endoplasmic reticulum, immature secretory granules, Golgi areas and on the plasma membranes, than in normal GH cells. Comparison of normal GH cells with tumour cells in undifferentiated acidophil adenomas demonstrated significantly larger volumes of nuclei, rough endoplasmic reticulum, Golgi fields, immature secretory granules and of the cell membranes, and also of nucleoli and of the mitochondria. Secretory granules and lysosomes were observed more frequently than in normal GH cells. In a comparison of both adenoma types, the well differentiated acidophil adenomas contained significantly larger volumes of the unorganized cytoplasm, secretory granules and of cell membranes, whereas more points were counted on the rough endoplasmic reticulum and on the mitochondria in undifferentiated acidophil adenomas. The differences between the normal GH cells and the GH cell in undifferentiated adenomas (mainly larger nucleoli, larger volumes of the rough endoplasmic reticulum and the lower volumes of secretory granules) indicate a higher secretory activity in the adenomas. The significant differences between the well differentiated and the undifferentiated adenomas (mainly the increased volumes of mitochondria and of the unorganized cytoplasm in the undifferentiated tumours) indicate a lower grade of differentiation and may be interpreted as signs of increased proliferation.

Key words: Pituitary adenomas – GH cells – Morphometry – Ultrastrucutre

Introduction

Classification of pituitary adenomas has to take into account not only the intracellular hormone content, but also the grade of differentiation. This is defined by the similarity of the adenoma cells to normal pituitary cells. Thus immunoctochemical methods have to be adopted for demonstration of hormone content (Kovacs et al. 1981; Mukai 1983; Saeger et al. 1986b) and plastic embedding is necessary for good structural preservation and a reliable examination of differentiation.

Other groups (Kovacs and Horvath 1986) have developed a very detailed classification of pituitary adenomas with many subgroups but we prefer a more simple classification, considering two grades of differentiation in addition to the hormone content.

In routine diagnosis it is often difficult to classify an adenoma as well differentiated or as undifferentiated (Saeger 1977; 1981; Riedel et al. 1985). The slight differences in structural features detected with the light microscope or even with an electron microscope, are interpreted qualitatively and to a certain degree, subjectively. The best method for exact analysis is morphometry on the

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** Dedicated to Prof. Dr. Klaus-Joachim Hempel on the occasion of his 60th birthday

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electron microscopical level which has been performed in some studies of pituitary adenoma (Tindall et al. 1982; Saeger et al. 1985; 1986a; Schottke et al. 1986).

We wished to study two randomly sampled groups of light microscopically classified pituitary adenomas (5 well differentiated acidophil GH cell adenomas (group 2), and 5 undifferentiated acidophil adenomas (group 3)), in acromegaly and to compare them with the normal GH cells from which GH secreting adenomas probably develop.

We used ultrastructural morphometry by the point counting method (Weibel et al. 1966; Saeger and Caselitz 1974) in order to detect slight but significant differences and to answer the question of whether or not this classification is justified.

Material and methods

10 pituitary adenomas, histologically classified as well differentiated aciophil GH cell adenomas ($n=5$) (group 2) defined by their distinct similarity to normal GH cells, or as undifferentiated acidophil adenomas ($n=5$) (group 3) defined by demonstrable acidophil granules and the lack of similaritiy to normal acidophil cells, (Table 1) were sampled randomly in a collection of more than 300 surgically removed pituitary tumours in acromegaly. Clinical data are listed in Table 1.

For comparison with normal structures, 3 pituitaries from patients with metastasizing cancer of the prostate were collected surgically (group 1) (Table 1).

After fixation in Bouin's solution, the greater portions of the specimens were embedded in paraffin and stained with haematoxylin-eosin, PAS, and performic acid-alcian blue-PAS-orange G. Immunocytochemical studies with the peroxidase-anti-peroxidase-method (Sternberger 1979) (diluted primary antiserum overnight at 4° C) were performed using the following primary antibodies: anti-GH (Kabi Vitrum GmbH, München FRG, dilution 1:100), anti-PRL (Serono Pharmazeutische Präparate GmbH, Freiburg, FRG, 1:200), anti-ACTH (Ferring Arnzeimittel GmbH, Kiel, FRG, 1:200), anti-TSH (Kabi Vitrum, 1:200), anti-LH (Kabi Vitrum, 1:250), and anti-FSH (Panchem Gesellschaft für chemische Produkte GmbH, Kleinwallstadt, FRG, 1:100).

For electron microscopic studies, small samples of each adenoma were fixed in glutaraldehyde for 2 h, postfixed in osmium tetroxyd and embedded in epon 812. Ultrathin sections (0.8 μ) were stained with uranyl acetate and lead citrate.

For ultrastructural morphometry, about 8 ultra-thin sections of each case were photographed in the electron microscope (Zeiss EM 9 S2) with a constant primary magnification (×4000) by random sampling. Final photographic magnification (size 17×18 cm) was 10500.

Table 1. Clinical and histological data

Case No.	Specimen No.	Histological diagnosis	Age (years)	Sex	Adenoma size	GH plasma levels μg/l	PRL plasma levels μg/l	Other pituitary hormones	Immunohistology					
									GH	PRL	ACTH	TSH	LH	FSH
1	S 169/76	(Cancer of prostate)	74	m	–	–	–							
2	S 76/77	(Cancer of prostate)	65	m	–	–	–							
3	S 80/79	(Cancer of prostate)	69	m	–	–	–							
4	S 26/79	Well differentiated GH cell adenoma	65	f	2 ps i	235.5	n	(↓)	+++	–	–	–	(+)	–
5	S 46/79	Well differentiated GH cell adenoma	44	f	1	68	n	n	++++	++	–	(+)	+	–
6	S 48/79	Well differentiated GH cell adenoma	57	f	1	30	32	n	+++	–	–	–	+	–
7	S 60)79	Well differentiated GH cell adenoma	34	m	1	15	n	n	++	++	–	–	(+)	–
8	S 65/79	Well differentiated GH cell adenoma	54	f	3 ss l	135	n	(↓)	++++	+	–	–	–	–
9	S 30/79	Undifferentiated acidophil adenoma	29	f	2 ss l	12	29	n	+++	–	–	–	–	–
10	S 38/79	Undifferentiated acidophil adenoma	58	f	2	32	21	n	+++	+++	–	–	+	–
11	S 40/79	Undifferentiated acidophil adenoma	27	m	2	50	n	(↓)	+++	–	–	–	–	–
12	S 50/79	Undifferentiated acidophil adenoma	48	m	3 ps i	60	44	(↓)	+++	++	–	–	(+)	–
13	S 61/79	Undifferentiated acidophil adenoma	48	m	2 i	39	54	(↓)	+++	+++	–	–	(+)	–

– = negative; (+) = very few adenoma cells positive; + = up to 10% of adenoma cells positive; ++ = 10–40% of adenoma cells positive; +++ = 40–80% of adenoma cells positive; ++++ = > 80% of adenoma cells positive

1 = micro < 10 mm; 2 = medium 10–20 mm; 3 = large > 20 mm; ss = suprasellar; ps = parasellar; i = invasive

(↓) = partial insufficient; n = normal

For morphometry, 132 photographs (37 from normal GH cells, 50 from well differentiated GH cell adenomas, and 45 from undifferentiated acidophil adenomas) were covered by a transparent coordinator screen with 1890 points for counting (squares 4 × 4 mm) according to the method of Weibel et al. (1966), Saeger and Caselitz (1974), Farquhar et al. (1978) and Mac Comb and Kovacs (1978).

Test points on nuclei, nucleoli, rough endoplasmic reticulum, Golgi fields, immature secretory granules, secretory granules, lysosomes, mitochondria, and cell membranes were counted. The points on the surrounding cytoplasm and of non-identifiable structures were classified as "unorganized cytoplasm".

Lysosomes were distinguished from secretory granules by their irregular shape, the heterogeneity of electron density and their generally larger size.

Intracellular spaces, capillaries, areas of necrosis, bleeding, and fibrosis were disregarded. The percentages of the numbers of test points for each oragnelle and the average values of the "volume densities" were calculated. The data of the three different groups were statistically compared (Wilcoxon's Test) in a Hewlett Packard minicomputer (9810 A calculator). Significant differences were interpreted at a level of $p < 0.05$.

Results

Ultrastructure

In normal GH cells the nuclei are oval or slightly lobate having small nucleoli at the periphery and moderate heterochromatin. (Fig. 1) The rough endoplasmic reticulum is sparsely developed. The Golgi areas are small. The mostly numerous secretory granules are very electron dense. Their diameters are about 400 nm. The mitochondria are monomorphic and oval. Lysosomes are mostly sparse.

In well differentiated adenomas (Fig. 2) the nucleoli contain more heterochromatin than those of the control group and seem to be enlarged. The centrally localized nucleoli are medium-sized and often doubled. The rough endoplasmic reticulum is moderately developed. Golgi fields have a medium or large size and are surrounded by sparse immature secretory granules. Mature secretory granules are numerous and diffusely arranged or mostly localized at the periphery. They have diameters up to 600 nm. Lysosomes vary in size, structure, lipid content, and numbers.

In undifferentiated acidophil adenomas (Fig. 3) the nucleoli are pleomorphic, demonstrating increased heterochromatin and enlarged nucleoli. The rough endoplasmic reticulum is well developed showing long parallel double membranes with many ribosomes. Free ribosomes are increased. The Golgi areas are medium-sized or in some cells enlarged. Secretory granules are sparsely arranged, varying in size and structure and having diameters between 120 and 800 nm. Mitochondria are numerous in some cells and slightly pleomorphic. Lysosomes are mostly sparse and small. Cellular membranes are often meandering.

Morphometry

The results of morphometry showed that the relative volume of the nuclei (Table 2) is between 14.2% in the normal GH cells and 23.6% in the undifferentiated adenomas. Standard deviations are high. Significant differences exist between the nuclear volume of normal GH cells and undifferentiated adenomas.

The relative volume of nucleoli (Table 2) is between 0.17% and 0.65%. It is significantly larger in the adenoma cells than in the normal GH cells, but there are no significant differences between the two adenomas types.

The relative volume of the unorganized cytoplasm (Table 2) lies between 37.1% and 41.8%. It is significantly larger in the well differentiated adenomas in comparison with the undifferentiated adenomas and with the normal GH cells.

The rough endoplasmic reticulum (Table 2) is most strongly developed in the undifferentiated adenomas (relative volume 18.8%) in comparison with the well differentiated adenomas (relative volume 13.3%) and the normal GH cells (7.0%). Differences between the two adenomas types are also significant.

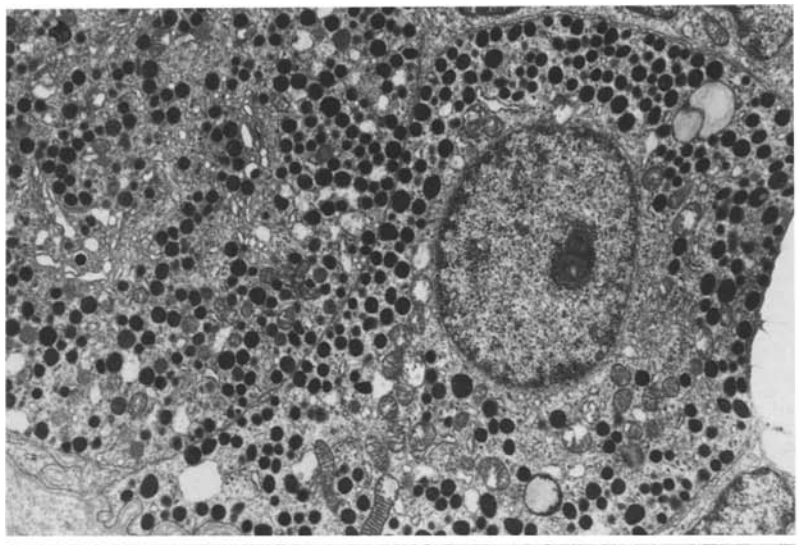
The Golgi areas (Table 2) have a relative volume between 2.7% and 5.6%. They were significantly larger in the adenomas than in the normal GH cells. Differences between the adenoma types are not significant.

Immature secretory granules (Table 2) with a relative volume of 0.13% to 0.65% were increased in the same way as the Golgi fields in the adenomas compared with the normal GH cells. Differences between the adenomas do not exist.

Secretory granules (Table 2) have the largest relative volume parts in the normal GH cells (21.4%). Differences from both adenoma types are significant. Furthermore, the well differentiated GH cell adenomas have significantly larger volumes of secretory granules than the undifferentiated types.

Analogous to the secretory granules, relative volumes of lysosomes (Table 2) (between 6.3% and 1.0%) are significantly larger in the normal GH cells than in the adenomas, but differences between the adenoma types are not detectable by quantitative methods. The undifferentiated adenomas have a significantly larger relative volume of mitochondria (Table 2) (7.3%), in comparison

1



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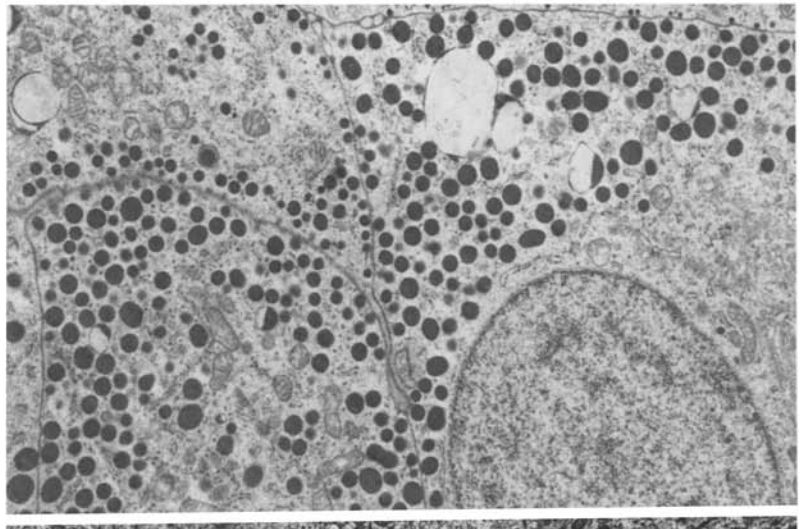

3

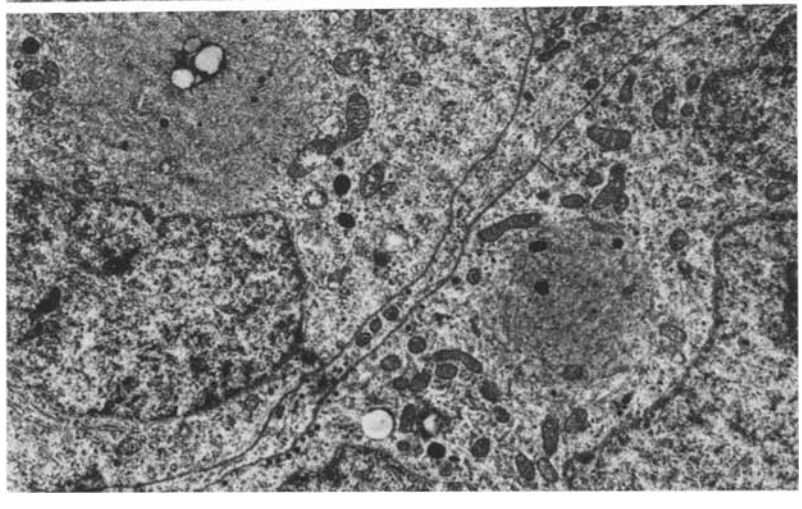

Fig. 1. Normal GH cells: Round nucleus, sparse rough endoplasmic reticulum, medium-sized Golgi field, many large secretory granules, sparse lysosomes. Magnification × 7220

Fig. 2. Well differentiated GH cell adenoma: Round nucleus, moderately developed rough endoplasmic reticulum, small Golgi field, many secretory granules, some lysosomes. Magnification × 7220

Fig. 3. Undifferentiated acidophil adenoma: Lobate nuclei, moderately developed rough endoplasmic reticulum, sparse differently sized secretory granules, some lysosomes, two fibrous bodies, many free ribosomes. Magnification × 7010

Table 2. Morphometric comparison of GH cells and GH producing adenomas. Mean values (%) and standard deviation (brackets) of relative volume parts of cell organelles

	Normal GH cell	Significant* difference	Well differentiated GH cell adenomas	Significant* difference	Undifferentiated acidophil adenoma	Significant* difference	Normal GH cell
Nucleus	14.23 (10.06)	n.s.	21.15 (8.60)	n.s.	23.65 (8.06)	>	14.23 (10.06)
Nucleolus	0.17 (0.30)	<	0.65 (1.67)	n.s.	0.31 (0.36)	>	0.17 (0.30)
Unorganized cytoplasm	38.94 (8.82)	<	41.83 (6.81)	>	37.06 (6.27)	n.s.	38.94 (8.82)
Rough endoplasmic reticulum	7.01 (3.52)	<	13.34 (3.73)	<	18.80 (3.59)	>	7.01 (3.52)
Golgi areas	2.72 (3.59)	<	4.05 (2.97)	n.s.	5.16 (3.69)	>	2.72 (3.59)
Immature secretory granules	0.13 (0.20)	<	0.65 (0.67)	n.s.	0.32 (0.21)	>	0.13 (0.20)
Secretory granules	21.39 (7.69)	>	8.24 (4.72)	>	3.25 (3.84)	<	21.39 (7.69)
Lysosomes	6.33 (4.94)	>	1.01 (2.32)	n.s.	0.73 (2.15)	<	6.33 (4.94)
Mitochondria	6.45 (2.75)	n.s.	5.88 (1.64)	<	7.34 (1.79)	>	6.45 (2.75)
Cell membranes	2.31 (1.72)	<	3.51 (1.15)	>	3.37 (1.21)	>	2.31 (1.72)

* $P<0.05$

n.s. = not significant

with well differentiated tumours (5.9%) and normal GH cells (5.4%).

Cell membranes (Table 2) with a relative volume between 2.3% and 3.5% are significantly more developed in the adenomas in comparison with normal GH cells. The well differentiated adenomas contain more than the undifferentiated.

Discussion

Pituitary GH cells and tumour cells of pituitary adenomas have been well documented in the literature at the light microscopical and electron microscopical level. Taking morphometry as the appropriate method and combining this technique with functional endocrinological (Table 1) and immunohistological data, we have specified the ultrastructural appearances morphometric analyses demonstrate distinct. And significant ultrastructural differences between the normal GH cells, the well differentiated GH cell adenomas and the undifferentiated adenomas.

When comparing GH cells and GH cell adenomas (Table 2), we measured a larger relative volume of nuclei, unorganized cytoplasm, rough endoplasmic reticulum, Golgi fields, immature granules, and lower relative volumes of secretory granules and lysosomes. Comparison between GH cells and undifferentiated adenomas revealed similar results. Larger volumes of nuclei, nucleoli, rough endoplasmic reticulum, Golgi fields, immature secretory granules, mitochondria and cell membranes and smaller relative volumes of secretory granules and lysosomes were found in the undifferentiated adenomas.

A higher secretory activity of the adenomas was indicated by the following features: larger nucleoli, development of the rough endoplasmic reticulum and of the Golgi fields, and a lower proportion of mature secretory granules and of lysosomes. These facts, assumed by others (Kinnman 1973; Schechter 1973; Adelman 1980; Horvath and Kovacs 1980; Trouillas et al. 1980; Kovacs and Horvath 1985; 1986) have been revealed on the quantitative level for the first time.

Comparison of well differentiated and undifferentiated adenomas (Table 2) revealed a significantly larger amount of cytoplasm and a larger relative volume of secretory granules and cell membranes but a smaller proportion of rough endoplasmic reticulum and mitochondria in the well differentiated GH cell adenomas. Similar results, although not

found by morphometry, were published for the differences between densely granulated GH cell adenomas (corresponding to the well differentiated acidophil adenomas in our classification) and the sparsely granulated GH cell adenomas which were in part comparable with our undifferentiated adenomas (Kinman 1973, Robert 1973; Trouillas et al. 1980; Kovacs and Horvath 1986). Landolt (1975) could not differentiate more than one adenoma type in his material of GH secreting adenomas. The increase of rough endoplasmic reticulum, swelling and cavitation of the mitochondria and sparseness of secretory granules were emphasized by Kovacs and Horvath (1986) for the sparsely granulated or undifferentiated adenomas and are compatible with our morphometric data.

In increased mitochondrial volume can be interpreted as the onset of oncocytic transformation and indicates the lower grade of differentiation. Nuclear signs of an increased proliferation (enlargement of nuclei and nucleoli) were not measurable in the undifferentiated tumours, in accordance with studies of Riedel et al. (1985), who compared the grading of acidophil adenomas by spreading, invasiveness and the rate of postoperative normalisation of GH levels. They found no significant differences for these variables.

From time-consuming morphometric studies, we have confirmed objective morphological similarities and differences between GH cells and both adenoma types, which justify the light microscopical classification of acidophil adenomas with acromegaly in routine daily diagnosis.

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